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THE ENTOLOMATACEAE OF THE PAKARAIMA MOUNTAINS OF GUYANA VIII: *ENTOLOMA ALDINOPHILUM* SP. NOV., A STRIKING SPECIES ASSOCIATED WITH *ALDINA INSIGNIS*

T. W. Henkel* & D. L. Largent

A new species of *Entoloma*, *E. aldinophilum*, is described from the central Guiana Shield region. The species' combination of features – including medium-sized, tricholomatoid, yellow basidiomata with intrinsically bright yellow trama, isodiametric, 6-angled basidiospores, and occurrence with the endemic ectomycorrhizal tree *Aldina insignis* (Benth.) Endl. (Fabaceae subfam. Papilionoideae) – is unique among *Entoloma* species worldwide.

Keywords. Agaricales, Basidiomycota, *Dicymbe*, ectomycorrhizal fungi, Guiana Shield, Neotropical fungi
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Introduction

The Entolomataceae (Agaricales, Agaricomycetes, Basidiomycota) is a speciose family of mostly lamellate mushrooms occurring in forests worldwide (Fries, 1838; Kotlaba & Pouzar, 1972; Noordeloos & Gates, 2012). Taxonomic concepts of genera within the family have varied among authors (e.g. Pegler, 1977; Noordeloos, 1992; Largent, 1994). Saprotrophic Entolomataceae species with diminutive statures and angled heterodiametric basidiospores occur in tropical forests worldwide (e.g. Horak, 1978; Largent *et al.*, 2020; Reschke *et al.*, 2022). Less frequently encountered in the tropics are species with large, tricholomatoid basidiomata and angled isodiametric basidiospores (e.g. Romagnesi & Giles, 1979; Largent *et al.*, 2008). Under alternative taxonomic schemes, species with these features would be placed in *Entoloma* s. str. *sensu* Largent (1994) or *Entoloma* subgen. *Entoloma sensu* Noordeloos (1981). *Entoloma* s. str. species are considered putatively ectomycorrhizal (ECM; e.g. Zerova & Rozhenko, 1966; Antibus *et al.*, 1981; Loree *et al.*, 1989; Agerer, 1997; Montecchio *et al.*, 2006; Kasuya *et al.*, 2010; Shishikura *et al.*, 2021).

Over the past 25 years, we have described numerous new species of Guiana Shield Entolomataceae among the saprotrophic genera *Alboleptonia*, *Calliderma*, *Inocephalus*, *Nolanea*, *Paraeccilia*, *Rhodocybe* and *Trichopilus* (Largent *et al.*, 2008; Aime *et al.*, 2010; Henkel *et al.*, 2010a, 2010b, 2014). Additionally, five species in *Entoloma* s. str. have been described from forests dominated by fabaceous ECM trees of the genera *Dicymbe* and *Aldina* (Largent *et al.*, 2008; Henkel & Largent, 2023).

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Here, we describe a new species that has the combination of features of *Entoloma* s. str. (= *Entoloma* subgen. *Entoloma sensu* Noordeloos, 1992), including a tricholomatoid stature, glabrous or innately fibrillose pileus surface, stipe apex that is > 4 mm broad, sinuate lamellae, subisodiametric to isodiametric, angled, pink basidiospores, broad pileus trama hyphae, minimal oleiferous hyphae, and abundant clamp connections (Largent, 1994). The new species appears to be an ECM associate of *Aldina insignis* (Benth.) Endl. (Fabaceae subfam. Papilionoideae) (Henkel *et al.*, 2002). Macromorphological, micromorphological and habitat information, and DNA sequence data for the ITS and 28S regions, are provided for the new species.

Materials and methods

The collections were made during the July 2024 rainy season in Guyana's Pakaraima Mountains adjacent to the Ireng River, within 1 km of a base camp located at 5°04'34"N, 59°58'22"W, 650 m, in a forest dominated by *Aldina insignis* (Henkel *et al.*, 2002). Methods for field description, microscopic analyses, and image capture followed Largent *et al.* (2008, 2020). Fungi were field-dried with silica gel. Colour designations follow Kornerup & Wanscher (1978), with colour plates noted in parentheses (e.g. 4A7). The type specimen was deposited in BRG (holotype) and HSC-F (isotype) (herbarium codes follow Index Herbariorum, updated continuously). Dried basidiomata were sectioned and rehydrated in 3% KOH.

A Nikon Eclipse Ci compound microscope (Nikon, Tokyo, Japan) with Lumenera Infinity 2 imaging software (Lumenera, London) was used to measure microscopic features following Largent (1994) and capture photomicrographs following Largent *et al.* (2020). In the taxonomic description, (x =) refers to mean dimensions of structures ± standard deviations, E refers to range of length of structures divided by their width, Q refers to the mean quotient of the length of structures divided by their width, and $n/3$ refers to the number of objects measured across three collections.

DNA was extracted from fungal tissue by heating with a Chelex extraction buffer (100 mM Tris pH = 8.5, 4% Chelex 100, 1% Triton X-100) at 99°C for 20 min, then freezing. After thawing, the resulting supernatant was used in PCR.

The PCR was performed in 25 mL reactions with 1 µL of DNA extract, 0.4 mM for each primer, 0.2 mM dNTP mixture, 5 µg of bovine serum albumin, and 0.5 U of OneTaq Hot Start DNA polymerase (New England Biolabs, Ipswich, Massachusetts, USA) in 1X OneTaq standard buffer. PCR conditions were 94°C for 30 s, followed by 36 cycles of 94°C for 15 s, 57°C for 30 s, and 68°C for 60 s, followed by a final extension at 68°C for 5 min. Primers ITS1F (Gardes & Bruns, 1993) and ITS4 (White *et al.*, 1990) were used to amplify the ITS1–5.8S–ITS2 region.

Sequencing was performed at Eurofins Genomics (Louisville, Kentucky, USA) using the same primers used for PCR. Forward and reverse reads were aligned using Genious 10.2.4 (https://www.geneious.com) and resolved manually, as needed.

Results

ITS (GenBank accession nos. PV920359, PV920360, PV920361) and 28S rRNA (GenBank accession nos. PV920362, PV920363, PV920364) sequences were generated from the type and additional collections in the present study. ITS sequences for the type (*T. Henkel* 11390) and the two additional specimens examined (*T. Henkel* 11406 and 11416) were identical.

Species description

Entoloma aldinophilum Largent & T.W.Henkel, **sp. nov.**

Similar to *Entoloma flavifolium* Peck in its basidioma size and tricholomatoid stature, bright yellow young lamellae, and hygrophanous pileus but differs in its olivaceous yellow pileus and stipe, yellow trama, and smaller, more smoothly angled basidiospores. – Type: Guyana, Region 8 Potaro-Siparuni, Pakaraima Mountains, Upper Ireng River Basin, 0.5 km NW of Kurutuik base camp at 5°04'34"N, 59°58'22"W, 650 m, on humic mat of forest floor under *Aldina insignis*, 14 vii 2024, *T. Henkel* 11390 (holotype BRG, isotype HSC-F [004459]). MycoBank 859223. GenBank accession nos.: ITS, PV920361; 28S, PV920364. **Figures** 1, 2.

Basidiomata tricholomatoid. *Pileus* 21–67 mm broad, 13–24 mm tall, initially broadly convex to broadly conic with low rounded umbo, with age planoconvex with low umbo and irregularly lobed margin, olivaceous yellow (3A7–3B7, 4B6–4B7, 4C6–4C7) throughout, with age lighter concolorous (3A5–3B5) over marginal 1/6, glabrous, slowly hygrophanous to lighter concolorous from disc outwards, marginal 1/3 faintly translucent-striate, this more pronounced with age, moist; surface under hand lens appressed radially fibrillose with minute erect elements throughout; margin crenulate to crenate with age; trama solid, bright yellow (2A4–A5–3A3–A5), unchanging, < 0.5 mm thick at margin, 0.5–1 mm centrally, 4–8 mm above stipe. *Lamellae* subthick, close, sinuate, initially bright yellow (2A4–A5–3A4–A5), with age concolorous with increasing pinkish tones, 1–2 mm tall at margin, 4–6 mm centrally, 2–5 mm at stipe; edges lighter concolorous, shallowly eroded, occasionally with basidiospore-accumulating insect webbing; lamellulae 3, occasionally 5, alternating long and short, 1–11 mm long. *Stipe* 49–88 mm long, 6–17 mm wide centrally, cylindrical over upper 1/2, widening to 15–20 mm over lower 1/2, then curving and tapering gradually or abruptly downwards to 6–10 mm wide at extreme base, yellow (2A3–A4–3A3–A4–A5), darker concolorous (2A5–3A5) where handled, finely longitudinally striate throughout; surface under hand lens of tightly appressed longitudinal fibrils; trama subsolid, bright yellow (2A6–A7), lighter concolorous over lower 1/2, unchanging, longitudinally fibrous, with narrow, partially hollow central core in upper 1/2; basal mycelium a scant to dense off-white to pale yellow mat surrounding extreme base. *Odour* slightly fungoid, of fresh clay. *Taste* instantly sweet, fading over 30 s to slightly nutty. *Spore print* dull pinkish tan in light deposit.

Figure 1. *Entoloma aldinophilum* Largent & T.W.Henkel, sp. nov. A, Tricholomatoid stature, range of basidioma sizes, olivaceous yellow pileus, yellow lamellae, and light yellowish-orange stipe (holotype, *T. Henkel* 11390); B, immature basidiomata with livid yellow-orange colours (*T. Henkel* 11406); C, longitudinal section of basidioma showing bright yellow pigment in the pileus and stipe trama (*T. Henkel* 11416). Scale bars, 10 mm. Photographs: Terry Henkel.

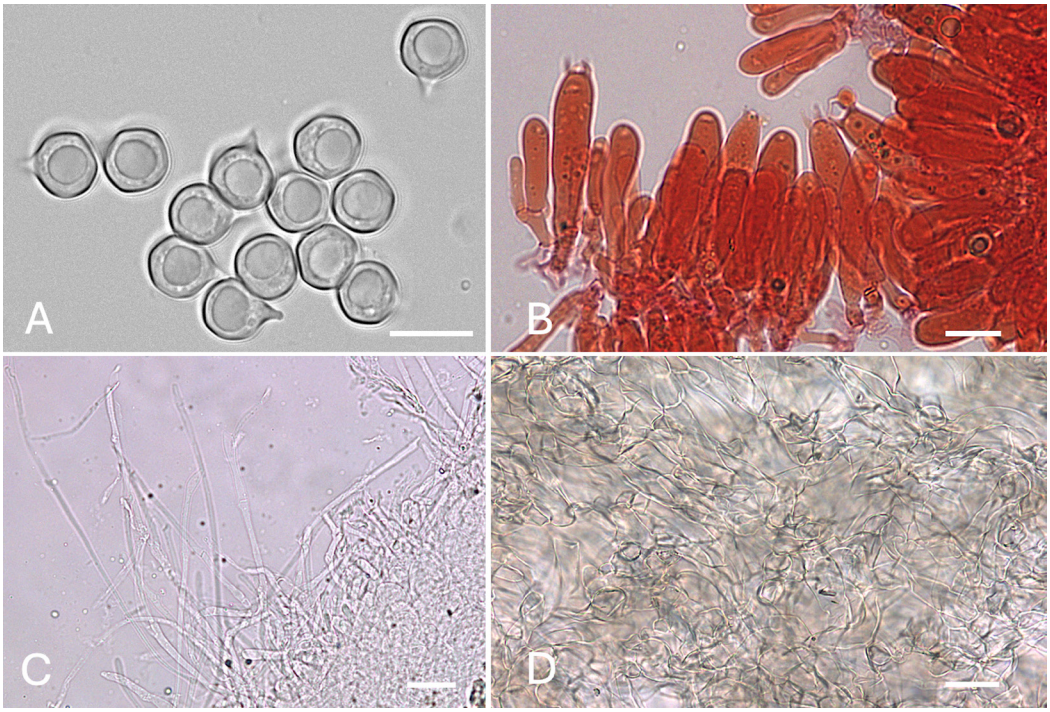

Figure 2. Microscopic features of *Entoloma aldinophilum* Largent & T.W.Henkel, sp. nov. A, Basidiospores; B, basidia and basidioles; C, pileocystidia and pileipellis; D, pileus trama. All photographs of the holotype, *T. Henkel* 11390, taken by D. L. Largent. Scale bars: A and B, 10 µm; C and D, 20 µm.

Basidiospores subisodiametric to isodiametric, 6-angled; angles initially smooth and indistinct, becoming distinct with maturation, usually with a single large globule, occasionally multiglobular, 5.8–8.0 × 5.2–7.7 µm (x = 6.8 ± 0.4 × 6.4 ± 0.4 µm; E = 1.0–1.2; Q = 1.1 ± 0.05); n/3 = 154). *Basidia* narrowly cylindro-clavate and tapered to a narrow base, with granules at maturity, 32.4–59.3 × 5.1–12.0 µm (x = 40.8 ± 4.5 × 8.6 ± 1.3 µm; E = 3.2–11.8; Q = 4.8 ± 1.1; n/3 = 99), 2 or 4 sterigmate; sterigmata 1.3–8.9 µm long (n = 193). *Cheilocystidia* and *pleurocystidia* absent. *Lamellar trama* 229.5–392.5 µm wide; hyphae subparallel and slightly interwoven; cells long and narrow, 3.2–78.4 × 0.7–13.8 µm (x = 33.5 ± 13.3 × 5.1 ± 1.9 µm; E = 0.6–39.9; Q = 7.3 ± 4.4; n/3 = 184). *Pileipellis* in radial section 15.3–110.3 µm thick (n/3 = 35), of tightly entangled, subpericlinal hyphae; pileocystidia suberect, scattered over disc, cylindrical to narrowly clavate, hyaline, 3.7–33.4 × 1.1–27.9 µm (x = 14.3 ± 6.8 × 5.4 ± 5.2 µm; E = 0.2–8.2; Q = 3.7 ± 1.7; n/3 = 92); subpellis clearly differentiated from pileus trama, of tightly entangled hyphae, 170–185 µm thick (n/3 = 35). *Pileus tramal hyphae* loosely entangled; cells in squash mounts relatively short and broad, 8.9–71.5 × 1.3–21.0 µm (x = 29.5 ± 14.6 × 9.0 ± 3.5 µm; E = 1.0–24.2; Q = 3.9 ± 3.0; n/3 = 138). *Stipitipellis* a cutis 11.1–108.8 µm thick (n = 21); caulocystidia infrequent

near stipe apex, cylindro-clavate, 8.1–50.7 × 2.2–14.1 µm (x = 27.1 ± 10.5 × 5.8 ± 2.9 µm; E = 2.1–10.1; Q = 5.1 ± 2.0; n/3 = 30). *Stipe tramal hyphae* subparallel and interwoven in longitudinal section; cells 11.3–92.9 × 2.5–17.5 µm (x = 49.8 ± 19.3 × 8.6 ± 3.4 µm; E = 1.6–15.3; Q = 6.33 ± 2.7; n/3 = 113). *Oleiferous hyphae* rare in the trama of the pileus, stipe, and lamellae. *Lipoidal globules* absent. *Pigments* yellow, present in all tissues, but soluble in water and 3% KOH and diffusing into mounting medium; hyphae thus appearing hyaline. *Clamp connections* abundant on bases of basidia and hyphae of the subhymenium, pileipellis, and trama.

Distribution. Known only from the type locality in the Upper Ireng River Basin of Guyana.

Habitat and ecology. In groups of 2–5 basidiomata on humic mat on sandy soils under *A. insignis*.

Etymology. *Aldina* and *-philus* (Greek, 'loving'), referring to the occurrence with the ECM tree *Aldina insignis*.

Proposed IUCN conservation category. Data Deficient (DD) due to inadequate information for the species' geographical distribution or population status in this remote, mycologically underexplored region.

Notes. *Entoloma aldinophilum* is recognised in the field by its medium-sized, tricholomatoid basidiomata with olivaceous yellow, smooth pileus, bright yellow, close lamellae, yellow, basally tapering stipe, and bright yellow, unchanging pileus and stipe trama. The species fits well into *Entoloma* s. str. based on its tricholomatoid stature, isodiametric 6-angled basidiospores, narrowly cylindro-clavate basidia, broad pileus tramal hyphae, and abundant clamp connections (Largent, 1994). The species is putatively ECM based on its inclusion in *Entoloma* s. str. and occurrence in forests dominated by ECM *Aldina* trees (Largent *et al.*, 2008).

Species of the Holarctic *Entoloma sinuatum* (Bull.) P.Kumm. complex (*Entoloma* subgen. *Entoloma* sect. *Entoloma sensu* Noordeloos, 2004) are characterised by tricholomatoid basidiomata and yellow lamellae (Morgado *et al.*, 2013), characters also seen in *E. aldinophilum*. Among these, the North American *Entoloma flavifolium* Peck most resembles *E. aldinophilum* in its olivaceous yellow-brown, rapidly hygrophanous pileus and yellow-ochre young lamellae but differs in its off-white (vs yellow) stipe, farinaceous (vs sweet) taste, off-white (vs bright yellow) trama, slightly more heterodiametric basidiospores (7.0–9.0 × 6.5–7.5 µm vs 6.7–8.0 × 5.9–7.7 µm; average Q = 1.2 vs 1.1) with 5–7 (vs 6) angles, abundant caulocystidia on the upper stipitipellis, lack of pileocystidia, and occurrence in *Quercus* woods of eastern North America (Morgado *et al.*, 2013). All other species in the *Entoloma sinuatum* complex differ fundamentally from *E. aldinophilum* in pileus colour and have a Holarctic distribution (Morgado *et al.*, 2013).

Among previously described Guiana Shield *Entoloma* s. str., only *E. olivaceocoloratum* Largent & T.W.Henkel resembles *E. aldinophilum* in its pileus colour and yellow, basally

tapering stipe, but it differs in its darker olive-brown, non-hygrophanous pileus and white immature lamellae (Largent *et al.*, 2008). Other sympatric *Entoloma* s. str. species from Guyana have white to brown pileus and stipe colours and white immature lamellae (Largent *et al.*, 2008; Henkel & Largent, 2023).

Elsewhere in the South American lowland tropics Entolomataceae species that bear some resemblance to *Entoloma aldinophilum* include *E. luteosplendidum* E.Horak & Cheype from French Guiana and Brazil, which has bright yellow basidiomata but differs in its mycenoid stature, heterodiametric basidiospores, and presence of hymenial cystidia (Horak & Cheype, 2007; Coimbra *et al.*, 2013). *Entoloma avilanum* (Dennis) E.Horak from eastern Brazil also has predominantly yellow colours but is mycenoid and has cuboidal, 4-angled basidiospores (Coimbra *et al.*, 2013). *Entoloma brasiliense* Blanco-Dios (= *Calliderma fibulatum*; Karstedt & Capelari, 2010) is microscopically similar to *E. aldinophilum* in its isodiametric basidiospores and abundant clamp connections but differs in its smaller basidiomata with a dark blue pileus and bluish-grey stipe (Karstedt & Capelari, 2010). The recently described *Entoloma hygropileum* L.F.Silva & Wartchow from northeastern Brazil has a tricholomatoid stature, isodiametric basidiospores, and abundant clamp connections but differs from *E. aldinophilum* in its brown, viscid to glutinous pileus and off-white stipe (Silva *et al.*, 2025). None of the South American Entolomataceae species treated by Rick (1939) or Horak (1978) combine the tricholomatoid stature and yellow colours seen in *Entoloma aldinophilum*.

Species of *Entoloma* subgen. *Entoloma* recently described from montane ECM *Quercus* and *Comarostaphylis* forests in Panama lack the yellow basidioma colours seen in *E. aldinophilum* (Reschke *et al.*, 2022). Among several Lesser Antillean species of Entolomataceae with tricholomatoid statures noted by Pegler (1983), only *Entoloma bakeri* Dennis vaguely resembles *E. aldinophilum* in its basidioma size and pale yellow pileus, but it differs in its white stipe and heterodiametric basidiospores.

Among tropical African species with tricholomatoid basidiomata with yellow colours, the Congolian *Entoloma flavofuscum* (Romagn.) Pegler differs from *E. aldinophilum* in its blackish-brown pileus and heterodiametric basidiospores (Romagnesi, 1956). The Cameroonian *Entoloma djaense* Largent & T.W.Henkel loosely resembles *E. aldinophilum* in its olivaceous brown pileus, yellowish-brown stipe, and isodiametric basidiospores but differs in its cylindrical, non-tapering stipe and versiform pileocystidia (Largent *et al.*, 2020). *Entoloma phaeum* (Romagn. & Gilles) Noordel. & Co-David from Gabon and Ivory Coast has large yellowish basidiomata but differs from *E. aldinophilum* in its decurrent lamellae and presence of cheilocystidia (Romagnesi & Gilles, 1979).

Australasian Entolomataceae with tricholomatoid statures and yellow basidioma colours include the Australian *Entoloma pamelae* Largent, which has a basally tapering stipe and similarly sized isodiametric basidiospores reminiscent of *E. aldinophilum*. *Entoloma pamelae* differs from *E. aldinophilum* in its lack of olivaceous tones in the pileus, off-white trama

that only yellows on exposure, and abundant cheilocystidia (Largent *et al.*, 2014). *Entoloma kewarra* Largent differs from *E. aldinophilum* in its pruinose bloom on the pileus and white trama staining greenish yellow on exposure (Largent *et al.*, 2014). From New Zealand and Tasmania, *Entoloma readiae* var. *sulphureum* (E.Horak) G.M.Gates & Noordel. has bright yellow young basidiomata but differs from *E. aldinophilum* in its diminutive, collybioid stature and heterodiametric basidiospores (Horak, 1973; Noordeloos & Gates, 2012).

There are several Indo-Malayan *Entoloma* s. str. species with tricholomatoid statures and yellow basidiomata. Among these, *Entoloma praeluteum* Corner & E.Horak is remarkably similar to *E. aldinophilum* in its similarly sized basidiomata with yellow trama, basally tapering stipe, and isodiametric basidiospores but differs in its shorter basidia (25–30 vs 32–59 µm), farinaceous (vs sweet) taste, and lack of pileocystidia (Horak, 1980). The Singaporean *Entoloma flavidum* (Massee) Corner & E.Horak, and *E. xanthomyces* Corner & E.Horak both diverge from *E. aldinophilum* in their broader (up to 140 mm) pileus, decurrent, white lamellae, and – in the case of *E. flavidum* – strongly heterodiametric basidiospores and presence of cheilocystidia (Horak, 1980).

Additional specimens examined. Guyana. Region 8 Potaro-Siparuni, Pakaraima Mountains, Upper Ireng River Basin, 0.6 km NW of Kurutuik base camp at 5°04'34"N, 59°58'22"W, 650 m, on humic mat of forest floor under *A. insignis*, 20 vii 2024, *T. Henkel* 11406 (BRG; HSCF [004460]). GenBank accession nos.: ITS, PV920360; 28S, PV920363; ~ 0.7 km NW of base camp, under *A. insignis*, 23 vii 2024, *T. Henkel* 11416 (BRG; HSCF [004461]). GenBank accession nos.: ITS, PV920359; 28S, PV920362.

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